


RNA-seq analysis

 Lluís Morey

Updated date: Sep 11, 2020

 An abbreviated version of this protocol was published in Science Advances in Jun 2020

Estrogen induces dynamic ER α and RING1B recruitment to control gene and enhancer activities in luminal breast cancer

DOI: 10.1126/sciadv.aaz7249

Detailed protocol

Hi,

In this paper we did not analyze eRNAs genome-wide.

eRNA analysis in T47D and MDA-MB-231 cells was performed in Chan et al. Nature Communications, 2018. See the method below:

Genome-wide identification of eRNA loci

For eRNA identification, the BEDtools window function was used to overlap H3K27ac and H3K4me1 peaks in a window of ± 200 bp. Next, BEDtools intersect was used with the option `-v` to discard any peak overlapping any exons from the hg19 reference genome (Gencode version 27), with additional 2 kb surrounding every exon. A ± 600 bp window at the center of the H3K27ac peak was used to calculate RPKM across the entire eRNA locus using total RNA-seq data, considering regions with eRNA expression to have RPKM > 0.3 . Next a cutoff of fold change > 2 or < -2 (shCTR versus shRING1B) was used to detected differential expression. eRNA loci was overlapped with super-enhancer regions or typical enhancer regions using BEDtools intersect.

Lluís

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Morey, L. (2020). RNA-seq analysis. Bio-protocol Preprint. bio-protocol.org/prep495.
2. Zhang, Y., Chan, H. L., Garcia-Martinez, L., Karl, D. L., Weich, N., Slingerland, J. M., Verdun, R. E. and Morey, L. (2020). Estrogen induces dynamic ER α and RING1B recruitment to control gene and enhancer activities in luminal breast cancer . Science Advances 6(23). DOI: [10.1126/sciadv.aaz7249](https://doi.org/10.1126/sciadv.aaz7249)

Copyright: Content may be subjected to copyright.